factors. To confirm mechanism, ECs were treated with recombinant VEGF at various concentrations and migration was measured.

Results: Hypoxia inhibited EC migration (0.87 vs 0.78 mm, $P < .05$) over 3 days. Coculture of ASC enhanced EC migration in both normoxic (0.87 to 0.93 mm) and hypoxic (0.78 to 1.02 mm; $P < .05$) environments. Media from cocultures in hypoxia contained significantly more VEGF (708.3 pg/mL) than normoxic cocultures (311.2 pg/mL) and ECs alone (28.9 pg/mL). The addition of VEGF to wounded EC cultures improved migration, but not to the extent of ASC coculture. Finally, hypoxia markedly increased expression of VEGF and slightly increased expression of bFGF in ASC.

Conclusions: These results demonstrate that (1) ASCs restore and enhance EC migratory function in a hypoxic environment, and (2) the effect is primarily due to secretion of VEGF by ASC in response to hypoxia, with other growth factors like bFGF likely playing a minor role. These results suggest that hypoxic preconditioning of ASC might be of value in enhancing their role in therapeutic angiogenesis to treat ischemic heart or limb conditions.

The Effect of Nitric Oxide and Statins on Thrombospondin-1-Induced Chemotaxis in Vascular Smooth Muscle Cells

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Objective: Vascular smooth muscle cell (VSMC) chemotaxis is important in intimal hyperplasia (IH). Nitric oxide (NO), a diffusible molecule that decreases VSMC chemotaxis to several growth factors, is protective against IH. Thrombospondin-1 (TSP-1), a matricellular glycoprotein that induces VSMC chemotaxis, acts antagonistically to NO in VSMCs. Statins exhibit direct and pleiotropic effects on VSMCs. We showed overnight treatment with lovastatin inhibited TSP-1-induced VSMC chemotaxis by mevalonate pathway inhibition and was Ras dependent. Hypothesis: Short-term statin treatment will inhibit TSP-1-induced VSMC chemotaxis and NO donors will enhance statin inhibitory effects.

Methods: Quiescent VSMCs were pretreated with serum-free media (SFM), hydrophobic fluvastatin (0.1μM, 0.2μM, 0.3μM, 0.5μM), or hydrophilic pravastatin (1μM, 2μM, 3μM, 5μM) for 20 minutes or 18 hours. Chemotaxis to SFM or TSP-1 (20 μg/mL) was determined with a modified Boyden chamber. Also studied was the effect of the NO donors, DETA-NONOate (0.1μM, 10μM, 100μM, 1000μM) or SNAP (0.1μM, 10μM, 100μM, 1000μM) on fluvastatin (0.5μM) or pravastatin (5μM) on TSP-1-induced chemotaxis. To determine the effect of acute statin treatment on Ras activation, cells were treated with fluvastatin (0.5μM) or pravastatin (5μM) and then exposed to TSP-1 or SFM. Active Ras was isolated using Raf-RBD agarose beads, and semiquantitation was performed by Western blot. Assays were performed in triplicate and analyzed by ANOVA.

Results: Pravastatin and fluvastatin inhibited TSP-1-induced chemotaxis (20 minute or 18 hour pretreatment, $P < .05$). DETA-NONOate and SNAP at high concentrations both impeded statin inhibition of TSP-1-induced chemotaxis ($P < .05$). Results of cytotoxicity assays were negative.

Conclusions: Hydrophilic and hydrophobic statins with acute treatment inhibited TSP-1-induced VSMC chemotaxis. This inhibition is likely a pleiotropic effect independent of HMG-CoA reductase and subsequent Ras prenylation; however, the role of HMG-CoA reductase remains to be determined. Further, high-dose NO reversed statin inhibition of TSP-1-induced chemotaxis, indicating NO and statin therapies in combination warrant further study.

Aged Rats Have Increased Neointimal Thickening and Ephrin-B2 Expression After Carotid Angioplasty

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Objectives: Although carotid angioplasty is associated with increased adverse events in elderly patients compared with younger patients, some animal models of carotid angioplasty have previously demonstrated only negative remodeling in aged rats compared with younger rats. Therefore, we examined the response to carotid artery angioplasty using a validated animal model of aging.

Methods: The right common carotid artery of young adult (6-month) or aged (22 to 24-month) male Fischer 344 rats was injured with a balloon, and the ipsilateral external carotid was ligated. The common carotid arteries were examined by histology and immunohistochemistry after 2 weeks.

Results: Aged and young adult rats had similar vessel areas after angioplasty (aged: 0.49 ± 0.02 mm^2; young: 0.4 ± 0.02 mm^2; n = 5-6; $P = .2$). However, aged rats had a significant reduction in the lumen area (0.18 ± 0.03 vs 0.24 ± 0.01 mm^2; $P = .02$), corresponding to increased neointimal thickening compared with young adult rats (aged: 0.15 ± 0.04 mm^2; young: 0.08 ± 0.03 mm^2; $P = .006$). Expression of Ephrin-B2, an arterial marker and ligand for Eph receptor signaling, was increased after angioplasty in both aged and young adult rats, but with higher amounts in aged animals (n = 4).

Conclusions: Aged Fischer 344 rats are an accurate model for carotid angioplasty in aged humans, with increased neointimal thickening after carotid angioplasty in aged rats compared with young adult rats. Increased Ephrin-B2 expression after carotid angioplasty suggests additional mechanisms of smooth muscle cell activation that may contribute to the increased neointimal thickening in aged rats and adverse events in aged humans undergoing carotid angioplasty.

Tissue Culture Analysis of an Intact Carotid Plaque: Is Cryoplasty an Option for Carotid Intervention?

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Objectives: Angioplasty imparts a cellular response to vascular smooth muscle cells (VSMCs) that contributes to intimal hyperplasia (IH). Previous cell culture manipulation has imputed that apoptosis follows rapid freezing, but no direct plaque sampling has confirmed this. An ex vivo carotid plaque model allows procedure-specific histologic and biologic assessment of metabolically active tissue.

Methods: An in-line, reconstructed carotid plaque model created after endarterectomy underwent either standard angioplasty (CAS) or dilation with the PolarCath (Boston Scientific) balloon (CRY). VSMCs were grown in tissue culture and harvested for interrogation with histologic staining, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL), and cell proliferation studies with bromodeoxyuridine (BrdU).

Results: Twelve plaques were successfully treated (6 CAS, 6 CRY). On histologic staining at 5 days, the mean number of mononuclear infiltrate cells (MNC) was 87/mm^2 for CRY vs 250/mm^2 for CAS ($P < .002$). The CAS group showed an initial positive staining for apoptosis at 24 hours, (mean concentration of apoptotic cells, 24/mm^2 [SD, 1-3]), which rapidly diminished over the 48 hours and 5 days to nearly zero. The CRY group showed an increased number of apoptotic cells at 24 hours (33/mm^2 [SD, 28-32]), and increased slightly over the following 48 hours and 5-day assays (36/mm^2 [SD, 33-38]; and 41/mm^2 [SD 38-44], respectively; see Fig). Although positive markers for BrDU were found in each specimen, quantitative analysis of nuclear mitotic activity among the specimens demonstrated no significant difference in cell proliferation between either group.

Conclusions: Carotid tissue treated with CRY vs CAS showed lower concentrations of MNC and a higher degree of sustained apoptosis. This suggests CRY may reduce IH lesions associated with endovascular carotid therapy by a mechanism not related to cell proliferation, but associated with apoptosis and inflammatory cell count reduction.